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Watermelon Quality Traits as Affected by Ploidy

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Abstract. **High-quality, high-phytonutrient watermelons [*Citrullus lanatus* (Thumb.), Matsum & Nakai] have strong market opportunities. To produce highly nutritious fruit in a seedless triploid market, the nature of phytonutrient accumulation as affected by ploidy must be understood. The present study performed on six field-grown watermelon diploid (2n) inbred lines, their induced autotetraploids (4n), and autotriploids (3n) determined the importance of ploidy on quality and nutritional content. Lycopene, total soluble solids (TSS), L-citrulline (hereafter referred to as citrulline), glutathione (GSH), weight, width, and length were measured in ripe fruit from one location. Our findings contradict some previous manuscripts, which did not use diploid inbred lines and their induced autoploidy relatives. Of the traits we analyzed that did not have a family-by-ploidy interaction (citrulline, GSH, weight, and width), we determined citrulline levels were not significantly affected by ploidy in five of six families nor was there a significant correlation when all family's citrulline values were averaged. Previous studies on field-grown fruit that did not use autoploidy lines suggested triploid fruit had more citrulline than diploid fruit. GSH was higher in autotriploid than in diploid or autotetraploid (95.0 vs. 66.9 or 66.7 $\mu g \cdot g^{-1}$ GSH, respectively). Additionally, we found an association with higher GSH in larger fruit. Autotriploid fruit were, in general, heavier and wider than diploid and autotetraploid fruit, and autotetraploid fruit were generally smaller than diploid fruit. Of the traits we analyzed that had a family by ploidy interaction (lycopene, TSS, and length), we determined within four families, ploidy affected lycopene concentration, but whether this interaction is positive or negative was family-dependent. These data suggest the triploid state alone does not give fruit higher lycopene concentrations. The mean TSS was higher in autotetraploid than in autotriploid, which was again higher than in diploid fruit averaged across families (10.5%, 10.2%, and 9.5% TSS, respectively); there was a family × ploidy interaction so the significance of this increase is affected by the triploid's parents. Lycopene and TSS had a slight positive correlation. Four of six families showed no statistical correlation between ploidy and length, and although mean length across family demonstrated smaller tetraploid fruit, the family-by-ploidy interaction demonstrates that this observation is family-dependent. Length and width correlate well with weight when combining data for all ploidy levels and when analyzing each ploidy separately. Length correlates more closely with width in autotriploid fruit than in diploid or autotetraploid fruit.**

Most of the U.S. watermelon market is seedless (triploid) fruit. Because so much of the market depends on triploids, it is important to understand the effect ploidy plays on quality traits for this crop. Traits such as size and yield have been extensively studied, but more complex experiments are needed to determine the effect ploidy has on quality traits and phytonutrient concentration. Boileau et al. (2003) indicated that lycopene alone is not responsible for reducing prostate cancer associated with increased tomato intake. This finding is not unexpected and demonstrates the importance of maintaining multiple nutritional compounds in fruits and vegetables for developing and maintaining complete nutrient potential through breeding.

Lycopene is a natural pigment synthesized by some plants and microorganisms but not by animals and humans. It is a potent antioxidant (Miller et al., 1996; Mortensen et al., 1997) found in significant amounts in only a few red-fleshed fruits including watermelon. Lycopene and other carotenoids are associated with a reduced risk of myocardial infarction (Kohlmeier et al., 1997) and certain types of cancer, especially prostate cancer (Gann et al., 1999; Giovannucci et al., 1995). Perkins-Veazie et al. (2001, 2006) demonstrated that triploid varieties typically have higher lycopene concentration than diploid varieties. However, this difference could simply reflect the intensive breeding efforts put into improving triploid varieties and may not reflect a ploidy effect itself. In fact, Leskovar et al. (2004) showed no ploidy group consistently higher in lycopene than the other. Liu et al. (2010) reported on nine sets of diploid inbred lines and their induced autotetraploids and autotriploids and demonstrated an increase in lycopene with increased ploidy levels (mean across all lines tested were diploid 44.1 $mg \cdot kg^{-1}$, autotriploid 49.8 $mg \cdot kg^{-1}$, and autotetraploid 50.9 $mg \cdot kg^{-1}$). However, this trend was not seen in all families and because the study was performed on greenhouse-grown fruit, it is difficult to stipulate if the same trend would be observed in field-grown fruit.

Perkins-Veazie et al. (2006) demonstrated that triploid varieties typically have slightly higher TSS concentration than diploid varieties. However, again, this study may reflect the intensive breeding pressure for high sugar in triploid varieties.

Amino acids have well-established individual roles in disease prevention. Arginine, an essential amino acid, functions as one of the 20 building blocks of proteins and in free form as a physiologic amino acid. Citrulline is a physiologic amino acid endogenous to most living systems. These amino acids are directly involved in clearing excess metabolic ammonia from the human body and are indirectly involved in cardiovascular function, immunostimulation, and protein metabolism (Curtis et al., 2005). Ingested arginine is cleared by hepatic cells, but citrulline is not and can serve as an arginine source in other parts of the body. Humans can effectively absorb citrulline from watermelon, which also increases plasma arginine levels (Collins et al., 2007; Mandel et al., 2005). Recently, subjects consuming watermelon or synthetic citrulline as a drink, combined with exercise, had reduced arterial blood pressure compared with a placebo (Figueroa et al., 2011). The heightened importance of watermelon as a source of bioactive compounds such as citrulline highlights the need for a better understanding of the genetic control of this

amino acid and other healthful compounds in watermelon. Watermelon is rich in citrulline (Tedesco et al., 1984), but the effect ploidy has on these compounds has not been adequately studied. Tarazona-Díaz et al. (2011) reported values on five lines (four of them triploid seedless varieties) grown at one location and determined that the seeded variety had less flesh tissue citrulline. In an earlier report (Rimando and Perkins-Veazie, 2005), six seeded varieties had on average less citrulline (1.8 mg·g^{-1} fresh weight) than did eight unrelated triploid varieties (2.4 mg·g^{-1} fresh weight). Unfortunately, in this study, a small sample size (three fruit for each variety) of unrelated varieties was used, and because the fruit tested were grown at different locations, it is difficult to assess the effect environment had on the fruit as well. It does appear that environment plays a role in citrulline concentrations (Davis et al., 2010–2011). Liu et al. (2010) reported that greenhouse-grown watermelon fruit from nine induced autotriploid hybrids had higher citrulline values than their diploid and induced autotetraploid parents, but these results were not statistically different. Additionally, it is not yet clear how greenhouse production affects citrulline as compared with the predominant field production method used in the United States.

GSH is a hydrophilic antioxidant tripeptide, which keeps reactive oxygen species from accumulating in cells and causing damage (Ames, 1983). Dietary absorption of GSH would be an ideal method by which to maintain or increase this important antioxidant; however, there is controversy as to whether GSH is absorbed to an extent that is helpful. Witschi et al. (1992) report supplementing volunteers with one dose of GSH did not increase plasma levels of this tripeptide. However, Hagen et al. (1990, 1991) and Hagen and Jones (1987) suggest that intact GSH could be absorbed from the gastrointestinal tract of rats. Various disease states (e.g., HIV/AIDS, chronic fatigue syndrome, certain cancers, and muscle wasting) have GSH deficiency. It is not clear in all of these diseases if the decreased GSH is the result of the disease itself or if pre-existing deficiencies of GSH exacerbate the disease by weakening the immune system and certain organs such as the brain and lungs (Balendiran et al., 2004; Bounous and Molson, 1999; Dröge and Holm, 1997; Gawryluk et al., 2011; Herzenberg et al., 1997; Richards and Roberts, 2000). GSH supplementation can improve survival rates in some diseases, for example HIV/AIDS (Herzenberg et al., 1997). Unfortunately, GSH can confer resistance to some chemotherapeutic drugs (Balendiran et al., 2004). Recent data showed decreased GSH levels (and thus likely increased oxidative stress) are associated with bipolar disorder and schizophrenia (Gawryluk et al., 2011).

There is no previously published comprehensive study on the range of GSH in watermelon, but there have been studies in other fruits. Wierzbicka et al. (1989) list GSH concentrations in commonly consumed foods, showing that red meat had the slightly higher levels than fruits and vegetables [the highest being broccoli, spinach, squash, and parsley (400 to 550 nmol·g^{-1} wet weight)] followed by grains and then dairy. This study also demonstrated that cooking decreased the amount of GSH. Because red meat is almost always cooked before consumption, the authors estimated the daily intake of GSH from meat and fruits and vegetables was almost equal. Davey and Keulemans (2004) demonstrated that apples with better storage properties maintain GSH levels better than poor storage apples during processing. Additionally, Bartolini et al. (2006) demonstrated that different developmental stages and storage conditions affected the amount of GSH found in apricots. Cheynier et al. (1989) demonstrated that variety plays an important role in GSH concentrations with a study of 28 grape varieties, which varied in their GSH concentrations from 56 to 372 μmole·kg^{-1}.

The change in shape of triploid watermelons has been noted for many years (Whitaker and Davis, 1962). Henderson (1977) performed a study using reciprocal triploid hybrids, two open-pollinated diploid lines and their autotetraploids. This study showed that the triploid state had a rounding effect on triploid fruit and the shape of the female parent affected how much rounding would occur.

When comparing effect of ploidy on a trait, it is imperative to use related inbred diploid and their autotriploid and autotetraploid lines. Otherwise, the intensive breeding efforts to improve triploid quality over the past few decades will likely skew the results toward triploid having better quality traits. Using diploid inbred lines, their induced autotetraploids and autotriploids eliminate the variety effect and thus phenotypic changes noted reflect the effects of ploidy only. The current study expands a preliminary trial by Liu et al. (2010) and was designed to measure effects of ploidy on watermelon phytonutrients and quality traits (lycopene, TSS, citrulline, GSH, weight, width, and length) under field-grown conditions. Six diploid inbred lines, their induced autotetraploids, and autotriploids were used.

Materials and Methods

Plant material. Noncommercial, experimental watermelon lines were supplied by coauthor, Dr. Wenge Liu, for this study. Six watermelon diploid inbred lines (lines were given numerical designations 16, 18 to 20, 22, and 24), their six induced autotetraploids, and the diploid and autotetraploid offspring (six autotriploids) were grown in Lane, OK (Bernow fine-loamy, siliceous, thermic, Glossic Paleudalf soil) in a randomized complete block design with four replications. Seed were planted in 96-cell speedling flats and were transplanted at the three- to four-true leaf stage. Ten transplanted seedlings were placed in each replication. Pre-plant fertilizer was added according to soil test results and plant care was provided as outlined in Motes and Cuperus (1995). Plants were transplanted onto raised beds two feet apart on 12-foot centers, were irrigated as needed through drip tape under black plastic (at the first signs of water stress), and fertigated bimonthly. All plants were grown during the summer of 2007 and were harvested throughout the season. All full-sized fruit (n = 570) were weighed and then cut through the ground spot. Length and width were measured on approximately half the fruit harvested and was only measured on fully ripe fruit with no misshapes (255 fruit). Length was measured from blossom end to stem end; width was measured from ground spot to sun spot. Quality traits were only analyzed for ripe fruit. Maturity was assessed by external and internal characteristics (i.e., waxiness, tendril death, TSS, firmness, and seed maturity). Heart tissue was measured for TSS for 558 fruit by squeezing juice onto an Atago PR100 digital refractometer (Bellevue, WA). Underripe samples were then disregarded and data from 441 fruit were analyzed for TSS concentration.

Lycopene quantification. Lycopene concentrations were determined for 441 watermelon samples using an UltraScan XE (Hunter Associates Laboratory, Inc., Reston, VA) as in Davis et al. (2003). Briefly, ≈50 g of sample was collected from the center of each fruit, pureed for 3 min with a Polytron PT 10-35 grinder (Kinematica AB, Lucerne, Switzerland) set at medium speed, and stored at –80 °C until analyzed. The instrument was standardized as per company specifications and blanked on an empty cuvette. Watermelon puree was mixed well to keep separation to a minimum; ≈20 mL of the sample was immediately poured into a 1-cm, 20-mL

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SR101A cuvette (Spectrocell, Oreland, PA). The sample was then scanned in the transmittance (TTRAN) mode under the following settings: the large reflectance port (1.00″), Illuminant at D65, MI Illuminant Fcw, and observer 10°. Absorbance at 750 nm was subtracted from absorbance at 560 nm to adjust for each sample's light scatter. Lycopene was estimated using the following equation:

$$=(\text{abs } 560 \text{ nm} - \text{abs } 750 \text{ nm}) \times 38.857) - 1.641$$

Citrulline quantification. Fish and Bruton (2010) and Liu et al. (2010) reported that the concentration of citrulline was highest at peak ripeness. Therefore, citrulline was analyzed from 103 ripe watermelon samples using a TLC plate method, which was a slight modification of a Brenner and Niederwieser (1960) method published in Davis et al. (2011). Citrulline was quantified on the basis of a citrulline standard (Sigma-Aldrich, St. Louis, MO). Samples were pureed as described previously. One milliliter of the liquid puree was centrifuged at 15,800 g_n for 10 min to remove debris. Supernatants were diluted to make a 20% solution in deionized water. Ten microliters of the diluents were loaded on a 20 × 20-cm silica gel matrix (200 µm layer thickness, 5 to 17 µm particle size) TLC plate (Sigma-Aldrich). The spots were air-dried and amino acids were resolved using a solvent (2:1:1 n-butanol:acetic acid:deionized water). Plates were developed with 0.2% ninhydrin in ethanol by baking at 95 °C for 5 to 10 min. Densitometric scans of the citrulline spots were visualized and calculated against standards using a Kodak Image station (Model 440CF; Eastman Kodak, Rochester, NY). Data are in $mg \cdot g^{-1}$ fresh weight, because 1 mL of watermelon puree is very nearly and consistently 1 g.

Total GSH quantification. One hundred twenty-five watermelon samples were analyzed using a GSH Colorimetric Detection Kit (BioVision Technologies, Mountain View, CA) using the total GSH company protocol. Briefly, 100 mg of pureed watermelon was added to 0.4 mL of GSH buffer and 100 µL of 5% 5-sulfosalicylic acid, mixed well, and centrifuged at 8000 × g for 10 min. Twenty microliters of the supernatant was added to a premixed and preincubated solution containing 20 µL nicotinamide adenine dinucleotide phosphate generating mix, 20 µL GSH reductase, and 120 µL GSH reaction buffer. The reaction was incubated at room temperature for 7 min before adding 20 µL of the substrate and incubated at room temperature for another minute. Absorbance was measured at 405 nm using a microplate reader (Bio-Rad 3550UV, Hercules, CA) and GSH concentrations were determined using a standard GSH calibration curve generated from company standards.

Data analysis. Data were subjected to analysis of variance using PROC GLM (Version 9.1.3; SAS, Cary, NC). Responses to main effects were determined and means separated using the Ryan-Einot-Gabriel-Welsch multiple F test. SAS's PROC CORR (Procedure for Pearson Product-Moment Correlation) analysis was used to determine the relationship of fruit weight, length, and diameter to watermelon quality traits. Linear least squares regression analyses were performed using the statistical component of Microsoft Excel® 2002 SP-2 software (Redmond, WA).

Results and Discussion

Factorial analysis (family × ploidy number) demonstrated significant interactions for the parameters lycopene, TSS, and fruit length (data not shown), so these parameters will not be discussed across families and ploidy number but will be discussed by individual families. There was no significant family × ploidy interaction for citrulline, GSH, weight, and diameter; therefore, these parameters are discussed by families averaged across ploidy numbers and ploidy number averaged across families.

Traits affected by ploidy. Four families exhibited a significant increase in TSS in autotriploid vs. diploid fruit (Table 1); additionally, autotetraploid fruit tended toward higher TSS than autotriploid fruit, although only one family line showed a statistically relevant difference. Two of the families did not demonstrate a significant difference for TSS as related to ploidy and, although the mean TSS was higher in autotetraploid than in autotriploid, which was again higher than in diploid fruit averaged across families (10.5%, 10.2%, and 9.5% TSS, respectively), there was a family × ploidy interaction so the significance of this increase is obviously affected by the family used to produce the autotriploid. These data support the Perkins-Veazie et al. (2006) data using unrelated diploid and triploid lines and demonstrate the importance of picking diploid and tetraploid lines, which produce high TSS triploids when developing new varieties.

The mean GSH concentration was higher in autotriploid fruit averaged across families than in the diploid or autotetraploid fruit (95.0 vs. 66.9 or 66.7 $\mu g \cdot g^{-1}$ GSH, respectively) (Tables 2 and 3). This may be the result of GSH levels' small positive correlation with larger fruit (see regression analysis below; Table 4). Because larger fruit have more GSH, and because triploids tend to be larger than diploids and tetraploids, this might account for higher GSH concentrations in triploids. There was such a high amount of variation within each family group that the difference was not statistically different between ploidy levels for four of the six lines, although all five of those family lines did exhibit a higher average GSH level in autotriploid fruit than in diploid or autotetraploid fruit. One of the lines demonstrated lower GSH in autotriploid fruit than in diploid fruit. Comparisons between families when all fruit from all ploidy levels were averaged did not show a significant familial difference. This is the first report on the range of GSH concentrations found in multiple watermelon varieties. The average concentrations of GSH across all fruit tested (70 $\mu g \cdot g^{-1}$) demonstrate that watermelon is a good source of GSH and is higher in this potent antioxidant than many grains, eggs, and dairy (Wierzbicka et al., 1989). Watermelon is higher in GSH than many fruits and vegetables, including apples, banana, pears, and green beans (≈10 $\mu g \cdot g^{-1}$)

Table 1. Impact of ploidy number (2X, 3X, and 4X) on total soluble solids (TSS) concentration within six watermelon families.[z]

Family no.	Ploidy number			Ploidy number		
	2X	3X	4X	2X	3X	4X
	Percent TSS			SD		
16	8.3 b[y]	9.9 a	10.5 a	1.3	1.2	1.0
18	10.9 NS	10.7 NS	11.1 NS	1.3	0.8	0.9
19	9.0 c	9.9 b	10.7 a	1.8	0.8	0.9
20	9.2 b	9.9 a	9.4 ab	1.0	1.1	0.9
22	9.6 b	10.1 a	10.7 ab	0.9	0.9	1.0
24	10.1 NS	10.5 NS	10.7 NS	1.8	0.9	1.0

[z]Comparisons were made only within families (rows) not between or among families.
[y]Any means within a row/family not followed by the same letter are significantly different at $P \leq 0.05$.
TSS = total soluble solids; NS = nonsignificant.

Table 2. Impact of ploidy number (2X, 3X, and 4X) on glutathione concentration within six watermelon families.[z]

Family no.	Ploidy number			Ploidy number		
	2X	3X	4X	2X	3X	4X
	Glutathione (µg·g–1)			SD		
16	63.7 NS	89.9 NS	84.0 NS	24.8	32.1	22.6
18	93.4 a[y]	61.1 ab	51.8 b	25.6	37.2	12.9
19	73.5 NS	85.2 NS	67.2 NS	16.9	27.7	17.8
20	51.1 NS	117.7 NS	60.1 NS	19.2	183.6	20.3
22	61.6 NS	79.9 NS	55.4 NS	30.2	31.1	15.7
24	56.2 b	109.1 a	79.6 ab	28.5	39.0	24.7

[z]Comparisons were made only within families (rows) not between or among families.
[y]Any means within a row/family not followed by the same letter are significantly different at $P \leq 0.05$.
NS = nonsignificant.

but is less than in parsley, spinach, broccoli (≈120 μg·g^{-1}), and red meats (50 to 400 μg·g^{-1}).

Autotriploid fruit were in general heavier and wider than their related diploid and autotetraploid fruit (Tables 3, 5, and 6). Autotetraploid fruit were generally smaller than diploid fruit. These results are consistent with previously published reports (Henderson, 1977). Autotriploid fruit from three family lines were significantly heavier and two significantly wider than diploid fruit. Additionally, fruit from four autotriploid lines were significantly heavier and one was significantly wider than autotetraploid fruit. For weight, one family difference between ploidy levels was not significant. Three families were not significantly different for width when compared across ploidy levels.

Traits unaffected by ploidy. Lycopene levels were not significantly affected by ploidy in two of the six families tested (Table 7), because there was a family-by-ploidy interaction; it is not statistically legitimate to discuss data for all families averaged, but it is interesting to point out that even when averaged across families, there was no noticeable difference (56.2 = diploid, 58.3 = autotriploid, and 59.7 = autotetraploid) in lycopene levels for the three ploidy levels. However, within four of the families, ploidy did affect lycopene concentration and this interaction seems to be family-dependent. In two families, 18 and 19, autotriploid fruit had significantly more lycopene than diploid fruit. In one family, 20, autotriploid fruit had more lycopene than in autotetraploid fruit and in another family, 22, this was reversed. Two families, 18 and 19, had significantly more lycopene in the autotetraploid than the diploid and in one family, 20, this was reversed. Several published articles claim lycopene is higher in triploid than in diploid varieties (Leskovar et al., 2004; Perkins-Veazie et al., 2001, 2006), but these studies did not look at related autoploidy watermelon lines. These studies do not consider the likely effect of extensive breeding efforts to improve quality of triploid watermelon over the past few decades, which likely confuses any real correlation of lycopene to ploidy. Our findings demonstrate that lycopene concentration correlates with ploidy, but the correlation can be positive or negative with increasing ploidy and the correlation is family-dependent. Our data agree with the Liu et al. (2010) report, which suggests that in greenhouse-grown watermelon fruit, ploidy affects lycopene concentrations, but how lycopene concentrations are affected is dependent on the family being tested.

Citrulline levels were not significantly affected by ploidy in five of six families nor was there a significant correlation when all families' citrulline values were averaged. However, in one family, 16, autotriploid and autotetraploid fruit had twice as much citrulline as diploid fruit (Table 8). These data contradict previous studies that did not use autoploidy lines and that suggested

Table 3. Family and ploidy analysis on fruit for L-citrulline, glutathione, weight, and width.[z]

	Avg across family							
	Citrulline		Glutathione		Wt		Width	
Family no.	(μg·g^{-1})	± SD	(μg·g^{-1})	± SD	(kg)	± SD	(cm)	± SD
16	2.7 b[y]	1.7	77.8 NS	26.5	2.9 c	1.2	16.3 c	2.1
18	4.2 a	2	71.3 NS	30.5	3.4 b	1.4	17.6 b	2.6
19	3.1 b	1.8	74.4 NS	20.9	3.3 bc	1.2	17 bc	2.2
20	3.4 ab	1.3	78 NS	111.3	3.9 a	1.6	18.6 a	2.1
22	4.4 a	2.2	62.9 NS	26.2	2.4 d	1.3	14.3 d	2
24	4.2 a	1.5	77.7 NS	35.1	3.2 bc	1.1	17.5 ab	1.8
	Avg across ploidy							
Ploidy no.	(μg·g^{-1})	± SD	(μg·g^{-1})	± SD	(kg)	± SD	(cm)	± SD
2x	3.4 NS	2	66.9 b	27.4	3.2 b	1.5	17.1 b	2.7
3x	4 NS	1.4	95 a	98.6	4 a	1.3	18 a	1.7
4x	3.8 NS	1.9	66.7 b	22	2.9 c	1.1	16.5 c	2.1

[z]Four traits with no significant family × ploidy interaction.
[y]Any means within a column not followed by the same letter are significantly different at $P \leq 05$.
NS = nonsignificant.

Table 4. Regression analysis of all traits tested against each other with all ploidys together, A, or with similar ploidy fruit analyzed separately, B, C, and D.

A) All fruit from all families							
	TSS	Lycopene	L-citrulline	Glutathione	Wt	Length	Width
TSS		0.2339	0.0847	0.0355	0.0355	0.0039	0.0098
Lycopene	0.2339		0.0293	0.0000	0.0062	0.0022	0.0388
L-citrulline	0.0847	0.0293		0.0334	0.0463	0.0335	0.0245
Glutathione	0.0355	0.0000	0.0334		0.0619	0.0108	0.0167
Weight	0.0355	0.0062	0.0463	0.0619		0.6338	0.8400
Length	0.0039	0.0022	0.0335	0.0108	0.6338		0.3809
Width	0.0098	0.0388	0.0245	0.0167	0.8400	0.3809	

B) Diploid (2n) fruit from all families							
	TSS	Lycopene	L-citrulline	Glutathione	Weight	Length	Width
TSS		0.2532	0.1408	0.0758	0.1336	0.0268	0.0593
Lycopene	0.2532		0.1591	0.0200	0.0000	0.0013	0.0069
L-citrulline	0.1408	0.1591		0.0838	0.1333	0.0746	0.0462
Glutathione	0.0758	0.0200	0.0838		0.1341	0.0061	0.3544
Weight	0.1336	0.0000	0.1333	0.1341		0.5557	0.8332
Length	0.0268	0.0013	0.0746	0.0061	0.5557		0.2779
Width	0.0593	0.0069	0.0462	0.3544	0.8332	0.2779	

C) Autotriploid (3n) fruit from all families							
	TSS	Lycopene	L-citrulline	Glutathione	Weight	Length	Width
TSS		0.1674	0.0975	0.0369	0.0647	0.0162	0.0005
Lycopene	0.1674		0.0002	0.0096	0.0009	0.0059	0.0151
L-citrulline	0.0975	0.0002		0.0781	0.0439	0.0019	0.0528
Glutathione	0.0369	0.0096	0.0781		0.1592	0.3326	0.1894
Weight	0.0647	0.0009	0.0439	0.1592		0.9134	0.7747
Length	0.0162	0.0059	0.0019	0.3326	0.9134		0.6756
Width	0.0005	0.0151	0.0528	0.1894	0.7747	0.6756	

D) Autotetraploid (4n) fruit from all families							
	TSS	Lycopene	L-citrulline	Glutathione	Weight	Length	Width
TSS		0.2553	0.0064	0.0250	0.0068	0.0000	0.0153
Lycopene	0.2553		0.0000	0.0033	0.0231	0.0213	0.1480
L-citrulline	0.0064	0.0000		0.0000	0.0015	0.0149	0.0000
Glutathione	0.0250	0.0033	0.0000		0.0255	0.2229	0.1374
Weight	0.0068	0.0231	0.0015	0.0255		0.7411	0.8820
Length	0.0000	0.0213	0.0149	0.2229	0.7411		0.3809
Width	0.0153	0.1480	0.0000	0.1374	0.8820	0.3809	

TSS = total soluble solids.

Table 5. Impact of ploidy number (2X, 3X, and 4X) on weight (kg) within six watermelon families.[z]

	Ploidy number			Ploidy number		
Family no.	2X	3X	4X	2X	3X	4X
	(kg)			SD		
16	2.5 b[y]	3.7 a	2.6 b	1.2	1.2	1.0
18	3.9 a	3.6 ab	2.9 b	1.7	1.3	0.8
19	3.0 b	4.0 a	3.0 b	1.2	1.1	0.9
20	3.7 NS	4.0 NS	3.7 NS	1.5	1.6	1.5
22	2.6 ab	3.5 a	2.1 b	1.7	1.4	1.0
24	3.0 b	4.1 a	3.0 b	1.3	1.0	1.0

[z]Comparisons were made only within families (rows) not between or among families.
[y]Any means within a row/family not followed by the same letter are significantly different at $P \leq 0.05$.

Table 6. Impact of ploidy number (2X, 3X, and 4X) on width (cm) within six watermelon families.[z]

Family no.	Ploidy number			Ploidy number		
	2X	3X	4X	2X	3X	4X
	cm			SD		
16	15.8 b[y]	17.8 a	15.9 ab	2.3	1.4	2.1
18	18.1 a	17.6 ab	16.3 b	2.9	2.0	1.4
19	17.0 NS	17.9 NS	16.8 NS	2.4	2.1	1.8
20	18.6 NS	19.5 NS	18.5 NS	3.0	0.7	2.2
22	14.6 b	18.5 a	13.7 b	2.7	—[x]	0.8
24	16.8 NS	18.5 NS	17.8 NS	1.9	1.8	1.6

[z]Comparisons were made only within families (rows) not between or among families.
[y]Any means within a row/family not followed by the same letter are significantly different at $P \leq 0.05$.
SD[x] not produced because there was only one value for 3x.
NS = nonsignificant.

Table 7. Impact of ploidy number (2X, 3X, and 4X) on lycopene concentration within six watermelon families.[z]

Family no.	Ploidy number			Ploidy number		
	2X	3X	4X	2X	3X	4X
	Lycopene ($\mu g \cdot g^{-1}$)			SD		
16	62.1 NS	63.0 NS	68.8 NS	14.5	10.4	13.1
18	63.5 b[y]	78.8 a	69.4 a	12.1	12.3	16.9
19	50.4 b	61.0 a	60.1 a	13.8	15.1	14.6
20	48.5 a	50.3 a	41.1 b	10.7	8.1	8.4
22	59.4 ab	50.9 b	65.4 a	14.7	6.3	9.7
24	53.3 NS	45.8 NS	53.2 NS	12.9	7.5	15.0

[z]Comparisons were made only within families (rows) not between or among families.
[y]Any means within a row/family not followed by the same letter are significantly different at $P \leq 0.05$.
NS = nonsignificant.

Table 8. Impact of ploidy number (2X, 3X, and 4X) on L-citrulline concentration within six watermelon families.[z]

Family no.	Ploidy number			Ploidy number		
	2X	3X	4X	2X	3X	4X
	L-citrulline ($mg \cdot g^{-1}$)			SD		
16	1.6 b[y]	4.1 a	3.1 a	0.9	1.4	1.9
18	4.9 NS	3.1 NS	5.0 NS	2.0	1.4	2.3
19	2.5 NS	4.6 NS	2.9 NS	1.3	1.9	1.9
20	2.5 NS	4.0 NS	3.4 NS	0.8	0.6	1.4
22	4.6 NS	3.2 NS	4.9 NS	2.6	0.6	2.4
24	4.1 NS	4.8 NS	4.1 NS	1.7	1.6	1.3

[z]Comparisons were made only within families (rows) not between or among families.
[y]Any means within a row/family not followed by the same letter are significantly different at $P \leq 0.05$.
NS = nonsignificant.

Table 9. Impact of ploidy number (2X, 3X, and 4X) on length (cm) within six watermelon families.[z]

Family no.	Ploidy number			Ploidy number		
	2X	3X	4X	2X	3X	4X
	cm			SD		
16	18.0 NS	20.1 NS	17.7 NS	3.9	2.4	2.3
18	20.4 NS	20.6 NS	18.6 NS	3.7	3.5	2.2
19	17.9 NS	19.6 NS	18.0 NS	3.0	2.7	2.5
20	21.6 a[y]	21.5 a	19.3 b	2.5	0.7	1.4
22	29.1 a	22.0 ab	15.7 b	6.9	—[x]	1.0
24	18.2 NS	21.0 NS	19.4 NS	2.5	2.0	1.8

[z]Comparisons were made only within families (rows) not between or among families.
[y]Any means within a row/family not followed by the same letter are significantly different at $P \leq 0.05$.
SD[x] not produced because there was only one value for 3x.
NS = nonsignificant.

autotriploid fruit had more citrulline than diploid fruit (Rimando and Perkins-Veazie, 2005; Tarazona-Díaz et al., 2011). Our results were similar to the article by Liu et al. (2010) that reported on greenhouse-grown watermelon fruit from nine induced autotriploid hybrids. Although they reported a higher citrulline value in autotriploid fruit than in their diploid and induced autotetraploid parents, there was no statistically relevant difference.

Four of six families showed no statistical correlation between ploidy and length and although mean length across family demonstrated smaller autotetraploid fruit, the family-by-ploidy interaction demonstrates that this observation is family-dependent (Table 9). In fact, two families showed smaller autotetraploid fruit when compared with autotriploid and diploid fruit as mentioned in a previous study (Henderson, 1977).

Trait correlations. As previously mentioned by Abd El-Hafez et al. (1985), Davis et al. (2008), and Perkins-Veazie et al. (2006), our data (Table 4) showed linear least squares regression analysis demonstrated a small but significant correlation between TSS and lycopene concentration. Regression values ranged from 0.1674 in autotriploid to 0.2553 in autotetraploids and 0.2339 when all fruit, regardless of ploidy, were analyzed. This regression correlation would be more striking if fruit at various stages of ripeness were analyzed because sugars and lycopene levels accumulate during ripening. So the correlation of TSS to lycopene concentration in ripe fruit is likely the result of two factors: 1) small indistinguishable levels of ripeness that are not detectable by our visual methods; and 2) sugars are important precursors for carotenoid synthesis and high sugar may be needed to drive high carotenoid development.

Interestingly, GSH demonstrated an association with fruit size (Table 4). Wider diploid fruit had more GSH (R^2 value = 0.3544) and longer autotriploid and autotetraploid fruit had more GSH (R^2 values = 0.3326 and 0.1374, respectively).

Linear least squares regression analysis demonstrated that length and width correlate well with weight when combining data for all ploidy levels and when analyzing each ploidy separately (Table 4). The R^2 values ranged from 0.5557 to 0.9134. This is expected because increased fruit size should result in increased fruit weight. Length correlates more closely with width in autotriploid fruit than in diploid or autotetraploid fruit (0.6756 vs. 0.2779 and 0.3809); again, this is not surprising because the 3n state has a rounding effect on watermelon fruit (Henderson, 1977).

Our results demonstrated that citrulline is unaffected by ploidy but that GSH, width, and weight are. There is a family-by-ploidy interaction for lycopene, TSS, and length, demonstrating the importance of screening triploid offspring for each diploid and tetraploid parent combination for these three traits. The presented data highlight the importance of parental line selection for triploid production. Proper choice and screening can promote triploids with good quality traits and phytonutrient concentrations.

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